Amendments to the Claims

Claim 1 (original): A nucleic acid construct comprising inverted repeat sequences of a transposable element and an origin of transfer, wherein the origin of transfer lies between the inverted repeat sequences, such that a transposition event involving the inverted repeat sequences will result in the origin of transfer being included in the resultant insertion at the transposition target site.

Claim 2 (original): A construct according to claim 1, wherein the inverted repeat sequences are of, or derived from, the inverted repeat sequences of, a transposable element that employs a non replicative transposition mechanism.

Claim 3 (original): A construct according to claim 1, wherein the inverted repeat sequences are, or are derived from, the OE and/or IE inverted repeat sequences of the transposon Tn5.

Claim 4 (currently amended): A construct according to claim 3, wherein the inverted repeat sequences have at least 70% identity with any one or more of the following sequences:

a) 5' CTGTC TCTTA TACAC ATCT 3' (SEQ ID NO: 1)
   3' GACAG AGAAT ATGTG TAGA 5' (SEQ ID NO: 2)

b) 5' CTGAC TCTTA TACAC AAGT 3' (SEQ ID NO: 3)
   3' GACTG AGAAT ATGTG TTCA 5' (SEQ ID NO: 11)

c) 5' CTGTC TCTTG ATCAG ATCTT GATC 3' (SEQ ID NO: 4)
   3' GACAG AGAAC TAGTC TAGAA CTAG 5' (SEQ ID NO: 12)

Claim 5 (original): A construct according to claim 4, wherein the inverted repeat sequences have at least 85% identity any one or more of said sequences a), b) and c).

Claim 6 (currently amended): A construct according to claim 3, wherein the inverted repeat sequences have the sequence:

5' CTGTC TCTTA TACAC ATCT 3' (SEQ ID NO: 1)

3' GACAG AGAAT ATGTG TAGA 5' (SEQ ID NO: 2)

Claim 7 (original): A construct according to claim 1, which does not encode a transposase.

Claim 8 (original): A construct according to claim 1, wherein the origin of transfer is an oriT which can be mobilised by the helper plasmids pUZ8002 and pUB307.

Claim 9 (currently amended): A construct according to claim 8, wherein the origin of transfer has the sequence:
CCGGGCAGGA TAGGTGAAGT AGGCCCACCC GCGAGCGGGT GTTCCTTCTT CACTGTCCCT TATTCGCACC TGGCGGTGCT CAACGGGAAT CCTGCTCTGC GAGGCTGGC (SEQ ID NO: 5),
or a variant thereof having origin of transfer function.

Claim 10 (original): A construct according to claim 1, which comprises a promoterless reporter gene located between the inverted repeat sequences.

Claim 11 (original): A construct according to claim 10, wherein the promoterless reporter gene is operatively associated with a ribosome binding site and the construct further comprises, upstream of the reporter gene and ribosome binding site and between the inverted repeat sequences, a translational stop sequence.

Claim 12 (original): A construct according to claim 1, which lacks an origin of replication.

Claim 13 (original): A construct according to claim 1, which is linear and consists essentially of the inverted repeat sequences and any sequences located therebetween.

Claim 14 (original): A vector including a construct according

to claim 1.

Claim 15 (original): A vector according to claim 14, which includes PCR primer binding sites and/or restriction sites for the amplification or excision from the vector of a linear nucleic acid consisting essentially of the construct.

Claim 16 (original): A method for mutagenising DNA of interest from a bacterial species, the method comprising:

a) contacting said DNA of interest with a nucleic acid construct according to claim 1, to form a transposition mixture;

b) incubating the transposition mixture under conditions suitable for transposition to occur, said contacting and incubating steps being performed other than within cells of said bacterial species;

c) transferring transposed DNA of said transposition mixture by conjugation from a donor bacterial cell into a host bacterial cell; and

d) incubating the host cell under conditions suitable for homologous recombination between the transposed DNA and the DNA of the host cell.

Claim 17 (original): A method according to claim 16, wherein the transposition mixture also includes a transposase.

Claim 18 (original): A method according to claim 17, wherein the transposase is, or is derived from, Tn5 transposase.

Claim 19 (original): A method according to claim 18, wherein the transposase is hyperactive mutant Tn5 transposase.

Claim 20 (original): A method according to claim 16, wherein the DNA of interest is contained in one or more circular DNA molecules.

Claim 21 (original): A method according to claim 16, wherein the construct is linear.

Claim 22 (original): A method according to claim 16, wherein the contacting and incubating steps (a) and (b) occur outside any bacterial cell, and the method comprises the further step (b1) of transferring the transposed DNA of the transposition mixture into the bacterial donor cell, prior to the conjugation step (c) into the host cell.

Claim 23 (original): A method according to claim 16, which comprises an additional step, before the conjugation step, of identifying the site in the DNA of interest at which a transposition event has led to an insertion.

Claim 24 (original): A method according to claim 16, wherein the DNA of interest is DNA from a bacterial library.

Claim 25 (original): A method according to claim 24, wherein the DNA from which the library is generated is from bacteria of the genus Streptomyces.

Claim 26 (original): A method according to claim 16, wherein the host cell is a pre germinated spore.

Claim 27 (original): A method according to claim 16, wherein the host cell is of the species or strain from which the DNA of interest originates.

Claim 28 (original): A method according to claim 16, wherein the donor cell is of a different cell type from the host cell.

Claim 29 (original): A method according to claim 16, which comprises an additional step (e) of detecting whether homologous recombination has occurred in the host cell.

Claim 30 (original): A method according to claim 29, wherein the additional step (e) comprises: detecting the loss in the host cell of a selectable marker that is borne by the DNA of interest; and detecting the retention of a selectable marker that is borne by the construct.

Claim 31 (original): A method according to claim 16, wherein the method comprises, prior to the conjugation step, an additional step of replacing part or all of the transposition-derived insert by a further step of homologous recombination, to remove sequences from the insert and/or to add sequences to the insert.

Claim 32 (original): A method according to claim 16, which is carried out simultaneously on several DNA molecules of interest, which are conjugated from different donor cells into different host cells, to produce a plurality of independently mutated host cells.

Claim 33 (original): A method for mutagenising DNA of interest of a bacterial species, the method comprising the steps of:

a) contacting said DNA of interest with a nucleic acid construct according to claim 1, to form a transposition mixture;

b) incubating the transposition mixture under conditions suitable for transposition to occur, said contacting and incubating steps being performed other than within cells of said bacterial species; and

c) storing transposed DNA of said transposition mixture for future use.

Claim 34 (original): A method for mutagenising DNA of interest of a bacterial species, the method comprising, following the production and storage of transposed DNA according to claim 33 above, the steps of:

a) transferring said transposed DNA by conjugation from a donor bacterial cell into a host bacterial cell; and

b) incubating the host cell under conditions suitable for homologous recombination between the transposed DNA and the DNA of the host cell.

Claim 35 (original): A host cell producible or as produced by the method of claim 16.

Claim 36 (original): A method of determining the effect of a genetic disruption, the method comprising culturing a host cell producible or as produced by the method of claim 16, and determining the effect of the disruption on the cell.

Claim 37 (original): Transposed DNA of interest producible or as produced by the process according to claim 33.

Claim 38 (original): Transposed DNA according to claim 37 contained in a bacterial cell or cells.

Claim 39 (original): A host cell producible or as produced by the method of claim 34.

Claim 40 (original): A method of determining the effect of a genetic disruption, the method comprising culturing a host cell producible or as produced by the method of claim 34, and determining the effect of the disruption on the cell.